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PRELIMINARY STUDY ON POTENCY OF COPROSTANOL AND *COLIFORM* BACTERIA IN SEMARANG COASTAL AREA

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ABSTRACT

Fecal coliform bacteria have been widely used as a biological indicator of sewage (domestic waste) pollution. However, as a biological indicator in urban coastal waters, it has disadvantage, in particular because of increased volume of industrial wastes that are toxic and heated, increase of salinity, and low dissolved oxygen. These conditions may affect the growth rate of most bacteria, including fecal coliform bacteria that becomes under representative in sewage pollution in urban coastal waters. It is necessary to find alternative indicator that can be used to better understand the sewage pollution in urban coastal waters. Many researchers have proposed coprostanol as a chemical indicator of sewage pollution. To understand the existence of coprostanol and coliform bacteria, a preliminary study has been done on water and sediment samples from the river, river mouth, and seawater of Banjir Kanal Timur Semarang coastal waters. The results showed that coprostanol could be detected in sediment from all sites, on the other hand coliform bacteria decreased with the increase of salinity, and were not detected in the seawater.

Key words: Coprostanol, *coliform*, coastal, pollution, sewage

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INTRODUCTION

Identification of sewage (domestic waste) pollution in the environment is important regarding health, esthetical, and other ecological reasons. Increased intensity and variety of human activities in coastal regions require specific environment conditions. Based on particular conditions, better understanding of the condition of sewage pollution in urban coastal waters become very important. The main problem of pollution is not only based on the toxicity of the pollutants (Alloway and Ayers 1994), but also the indirect impact to the environment quality. Pollution of organic substance, such as domestic wastes, affects the dissolved oxygen into insufficient condition for the organism, as well as contains a lot of other materials that are hazardous wastes, such as pathogen organism.

Coliform bacteria have been widely used as a biological indicator of sewage pollution. To determine each pathogen organism directly is not easy and sometimes is expensive, so that in the purpose of water quality management, it is necessary to have an indicator. *Coliform* bacteria have been used as an indicator

because they are found abundant in the fecal of human and animal, and in general are relatively easy to determine. The occurrences of *coliform* bacteria indicate the contamination of domestic wastes, therefore the existence of pathogen organism should be concerned (Dutka *et al*. 1974, Chapra 1997). However, using *coliform* bacteria as an indicator of domestic waste pollution in the environment with high environmental stress, such as urban coastal waters, has created other problems that may be affected by several factors: these are a) the increase of salinity from freshwater to seawater, b) increase of industrial wastes that are toxic and heated, and c) low dissolved oxygen (Walker *et al.* 1982, Bartlett 1987). These conditions may affect the growth rate of bacteria, so that the existing fecal *coliform* bacteria are under representative in sewage polluted urban coastal waters. To solve these problems, several researchers proposed coprostanol as a chemical indicator of sewage pollution (Hatcher *et al.* 1977, Hatcher and McGillivary 1979, Brown and Wade 1984, Düreth *et al.* 1986, Holm and Windsor 1990, Coakley and Poulton 1991, Coakley *et al.* 1992, Bachtiar 1993, Bachtiar *et al.* 1996, Jeng and Hang 1994, Jeng *et al.* 1996, Chan *et al.* 1998).

Coprostanol (5β-cholestan-3β-ol), a derivative of cholesterol by intestinal microorganism, is dominant fecal sterol in human faces (40-60 % of total sterol), and was also detected in mammal and chicken, but it was not produced by marine organism (Walker *et al.* 1982). The existence of coprostanol in coastal water environment indicates that domestic wastes have reached that area. Coprostanol had been used to indicate and to trace the domestic wastes, and the results showed that coprostanol has a high performance as an indicator and a natural tracer of domestic wastes However, all of the studies had been done in high latitude regions. To use coprostanol in Indonesia, it is necessary to well understand the existence and persistence of coprostanol. It is because as organic material, coprostanol will be degraded in the environment, especially in tropical region. Based on that fact, the existence of coprostanol and *coliform* bacteria in Banjir Kanal Timur Semarang coastal waters was examined.

MATERIAL AND METHODS

Sampling

Sampling of water and sediments were carried out at 5 stations in three environmental conditions: namely a) rivers, b) river's mouth, and c) seawater, of Banjir Kanal Timur Semarang coastal waters. There were three stations in the river environment: Banjir Kanal Timur (BKT), Kali Tambak Lorok (KTL), and Kali Tenggang (KT); one station was in river's mouth environment, and one station was in seawater environment. Six liters of water samples were collected for each station by using Niskin Bottle water sampler at 10 cm below the surface. One liter was prepared for *coliform* analysis. Surface bottom sediment samples were collected using van Veen Grab sampler, and sampled from the top to 2 cm of surface sediment. The samples were immediately put in dark bottle and stored in a cooler (5°C) during the fieldwork. Further all samples were immediately put into cold storage at 5°C until analysis.

Coprostanol Analysis

Coprostanol analyses were done for both sediment and water samples. Five liters of water samples for each station were pumped through the glass fiber filter paper (2.0 μm, Ahlstrom) using a hand vacuum pump to get the suspended material for coprostanol analysis. The sediment and suspended materials were prepared for Gas Chromatography (GC) analysis as follows (Bachtiar 2002):

a. Soxhlet extraction
Sediment and suspended sediment samples were dried using freeze-dried method. A minimum of 5 gram dried sample was needed for extraction. The samples were extracted in benzene:methanol (1:1, v/v) in soxhlet apparatus for 24 hours. Heptadecanol was added as internal standard to the extract. Extracted sediments were dried in the oven (60°C) for several days. The weights were continuously recorded until their values have no longer changed.
b. Evaporation
The extracts were evaporated until near dryness by using rotary evaporator.
c. Saponification
The extracts were dissolved in benzene:methanol (1:1, 2 ml, 3-4 times) in a 50 ml centrifuge tube and were then saponified with 5 ml methanolic KOH (0.5 N KOH in 95% methanol, 5 % H_2O). The tube was placed in boiling water bath for 20 minutes and allowed to cool.
d. Extraction
The saponification was followed by extraction by using n-hexane (5 ml) 4 times. The tubes were centrifuged for few minutes. The top organic phase was transferred into a pear shape flask (50 ml) using a long pipette. Methanol phase was discarded.
e. Fractionation
Extract lipids were fractionated using silica gel (deactivated with 5 % water) in chromatography column. Less polar lipids were fractionated using 40% hexane in chloroform, and fractions that contain sterol were isolated with 10 % methanol in chloroform. The fractions were collected in 9.5 dram vials, and stored in a fridge until preparation for Gas Chromatography (GC) analysis.
f. Sample Preparation for GC
Sterol fractions were evaporated just to dryness, and later the samples were transferred to HP septum–capped vial using 2 x 0.5 ml Heptane. BSTFA (Bis(trimethylsilyl)-trifluoroacetamide)
100 µl was added, and then the samples were heated at 130 °C for 15 minutes to make them more responsive on the GC capillary column. Finally, the samples were allowed to cool. Once cooled, the samples were ready for GC analysis.
g. Gas Chromatography
Analyses of coprostanol were carried on Hitachi 263-50 Gas Chromatography with an SE-30 column capillary, and standard flame-ionization detector (FID). Nitrogen was used as carrier gas (50 ml/minute). The Gas Chromatography was programmed that the injector and detector was 300°C, and the oven was 150°C – 280°C with increasing 5°C/minute. Coprostanol concentration was calculated based on relative response factor (RRF) from a reference solution containing coprostanol standard and reference standard. RRF was determined by using the following formula (Telford *et al*. 1993):

$$RRF = \frac{\mu g \text{ coprostanol in standard}}{\text{Area coprostanol in standard}} \times \frac{\text{Area reference standard}}{\mu g \text{ reference standard}} \quad (1)$$

Based on formula (1), coprostanol concentration in samples was determined by using following formula:

$$\mu g \text{ coprostanol} = \frac{RRF \times \text{Area coprostanol} \times \mu g \text{ Internal Standard}}{\text{Area Internal Standard}} \quad (2)$$

The response of internal standard (IS), reference standard, and coprostanol was the area of IS, reference standard, and coprostanol, that are determined from the GC output.

Total *Coliform* Analysis

Coliform analyses were done only for water samples. The method that was used for coliform analysis generally based on the multiple-tube fermentation technique through presumptive-confirmed or completed test (Greenberg *et al.* 1992). In this analysis three tubes were used per dilution.

a. Presumptive test

Lauryl tryptose broth culture medium was used in this test. Before sterilization, sufficient medium was dispensed in fermentation tube with an inverted vial, to cover the inverted vial at least one half to two–thirds after sterilization. Three tubes were prepared for each dilution (10 ml, 1 ml, and 0.1 ml). Each tube was inoculated in a set of three with replicate sample volume, and mixed test portion in the medium by gentle agitation. Inoculated tubes were incubated at 35 ± 0.5°C . After for 24 ± 2 hours, each tube was swirled gently and examined for heavy growth, gas, and acidic reaction (shade or yellow colour), and if no gas or acidic growth had formed, and reincubate and reexamine at the end of 48 ± 3 hours. The presence or absence of heavy growth, gas, and acidic reaction was recorded. Production of gas or acidic growth in the tubes represented a positive presumptive reaction. The tubes with a positive presumptive reaction were submitted to the confirmed test.

b. Confirmed test

Brilliant green lactose bile broth was used in confirmed test. Before sterilization, sufficient medium was dispensed in fermentation tube with an inverted vial, to cover the inverted vial at least one half to two–thirds after sterilization. All primary tubes showed heavy growth, any amount of gas, or acidic growth within 24 hours of incubation were submitted to the confirmed test. Tubes that showed active fermentation or acidic growth at the end of a 48 hours incubation period were submitted to the confirmed phase. The positive presumptive tubes were gently rotated to resuspend the organisms. With sterile metal loop 3 mm in diameter, one loopful of culture was transferred to a fermentation tube containing brilliant green lactose bile broth. The tubes were incubated at 35 ± 0.5°C for 48 ± 3 hours. Formation of gas in any amount in the tube at any time within 48 ± 3 hours has characterized a positive confirmed phase. The coliform density was calculated in terms of the Most Probable Number (MPN)/100 ml.

Fecal *Coliform* Analysis

EC medium was used for fecal *coliform* analysis. Before sterilization, sufficient medium was dispensed in fermentation tube with an inverted vial, to cover the inverted vial at least one half to two–thirds after sterilization. The positive confirmed tubes were gently rotated. With sterile metal loop 3 mm in diameter, one loopful of culture was transferred to EC broth. Inoculated EC broth tubes were incubated in a water bath at 44.5 ± 0,2°C for 24 ± 2 hours. Gas production with growth in an EC broth culture within 24 hours or less was considered a positive fecal coliform reaction. The fecal *coliform* density was also calculated in terms of the Most Probable Number (MPN)/100 ml.

RESULTS AND DISCUSSION

There are three streams, Banjir Kanal Timur (BKT), Kali Tambak Lorok (KTL), and Kali Tenggang (KT), which come into the Banjir Kanal Timur Semarang coastal waters together through the same river's mouth. The location was selected because along of those streams are mostly slum areas, where domestic wastes were dumped directly into the water body of the streams. In the surrounding area, there are many activities, such as industries, harbours, electrical power plant, fisheries, aquacultures, and recreation. The wastes of

all these activities increase the environmental stress in Semarang coastal waters, and affect the water quality. However, each activity usually requires a specific environmental quality.

The data of field measurement of water quality parameters and the results of coprostanol and *coliform* analyses are listed in Table 1 and plotted in Figure 1 and Figure 2. During laboratory analyses, the sample of Kali Tenggang (KT) was lost, therefore the river data only from Banjir Kanal Timur (BKT) and Kali Tambak Lorok (KTL). Coprostanol was detected in all of the sediment samples, but not detected in all of water samples. There are two possibilities that coprostanol was not detected in water samples. First, the amount of suspended samples was less than 5 g (1.5 – 2.7 g), the minimum amount required. Second, the accuracy of the equipment used was only in ppm, so low concentration could not be detected.

Table 1. The Results of Coprostanol and *Coliform* Bacteria Analyses

No	Station	Coprost. conct. in sediment (μg/g)	Coprost. Conct. in water (μg/g)	MPN Total *Coliform* (/100 ml)	MPN Fecal *Coliform* (/100 ml)	Temp. (°C)	Sal. ($^0/_{00}$)	DO (mg/l)	pH
1.	BKT	20.70	-	>2,400	>2,400	28.6	10.0	3.7	8.0
2.	KTL	15.05	-	1,100	1,100	28.2	9.4	4.6	7.8
3.	KT	n.a	n.a	n.a	n.a	28.7	9,6	3.9	8.2
4.	RM	1.15	-	460	150	29.7	19.7	6.3	7.9
5.	Sea	12.71	-	-	-	30.8	32.4	6.7	8.1

Note: BKT = Banjir Kanal Timur KT = Kali Tenggang * in sediment samples
KTL = Kali Tambak Lorok RM = River's Mouth ** in water samples
n.a = not available

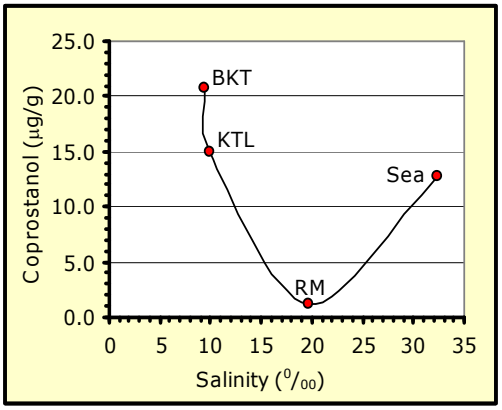

Fig. 1. Coprostanol concentration in rivers (BKT and KTL), river's mouth (RM), and the sea.

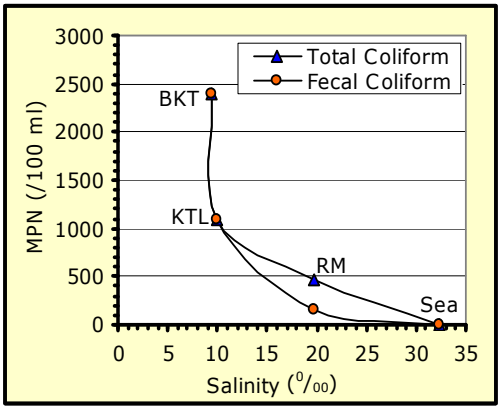

Fig. 2. *Coliform* concentration in rivers (BKT and KTL), river's mouth (RM), and the sea.

The highest concentration of coprostanol was detected in sediment of the river environment, in Banjir Kanal Timur (20.70 μg/g) and followed by Kali Tambak Lorok (15.05 μg/g). In the sediment of river's mouth, coprostanol concentration was decreased (1.15 μg/g), and was increased again in the seawater station (12.71 μg/g). The c*oliform* bacteria (total and fecal *coliform*) were detected at high concentration (> 2,400 MPN/100 ml) in water sample of Banjir Kanal Timur, and followed by water sample of Kali Tambak Lorok (1,100 MPN/100 ml). In the river mouth where the salinity was 19.7 $^{o}/_{oo}$, the total *coliform* was decreased to 460 MPN/100 ml, and the fecal *coliform* became 150 MPN/100 ml. In the sea station where the salinity was 32.4 $^{o}/_{oo}$, both total and fecal *coliform* were not detected. This fact indicates that: a) coprostanol existed in the three environmental conditions (river, river's mouth, and seawater), b) increase of salinity is the main factor that affects the presence of coliform bacteria in river's mouth and seawater. Bartlett (1987) found that increased salinity affected the osmotic pressure of bacteria cell, and in particular condition it affected the mortality rate of bacteria. Beside that increased volume of industrial wastes that are toxic most possibly affect the occurrence of *coliform* bacteria in the river's mouth and seawater of Banjir Kanal Timur Semarang coastal waters. Takarina and Bachtiar (2001) found that on sediments of Semarang coastal waters contain of high concentration of heavy metal: Cu (106 ppm), Cr (99.8 ppm), Pb (74.2 ppm), Zn (236 ppm), and based on geochemical fractionation of heavy metals they found that dominantly because of anthropogenic effects.

Based on coprostanol and *coliform* data show the agreement that Banjir Kanal Timur is the main source of domestic waste. It may be caused by the length of Banjir Kanal Timur (14.25 km), therefore it receives higher input of domestic wastes compared to Kali Tambak Lorok (6.50 km) and Kali Tenggang (5.20 km). Banjir Kanal Timur receives input from Kali Kedungmundu, Kali Bajak, and kali Candi (Bachtiar 2002). Domestic waste discharge of Banjir Kanal Timur (including Kali Tambak Lorok) was 4,690.7 m^3/day, and Kali Tenggang was 2,401.1 m^3/day (DPU Cipta Karya 1996).

CONCLUSION

The results showed that coprostanol could be detected quantitatively in the sediment of three environmental conditions: river, river's mouth, and seawater. On the other hand, the density of total *coliform* and fecal *coliform* bacteria in the water column decreased with the increase of salinity, and was not detected in the seawater. This fact indicates that to better understand of domestic waste pollution, especially in the environment with high environmental stress, such as urban coastal waters, it is necessary to use alternative indicator.

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